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Solid-state and solution-phase conformations of pseudoproline-containing dipeptides

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Abstract
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Keywords
pseudoproline, solid, containing, state, dipeptides, solution, phase, conformations, CMMB

Disciplines
Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

Authors

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Solid-State and Solution-Phase Conformations of Pseudoproline-Containing Dipeptides


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Introduction

Pseudoprolines ($\psi^{R,R'}$ pro) are oxazolidine or thiazolidine derivatives of serine, threonine, and cysteine residues that form on cyclocondensation of the amino acid with an aldehyde or ketone (Fig. 1). Their five-membered ring structure is reminiscent of a proline residue. These modified amino acid residues were introduced by Mutter and coworkers as temporary protecting groups for peptide synthesis and were found to exert a pronounced effect on the peptide backbone conformation. These structural differences are attributed to crystal-packing interactions.

![Fig. 1. Synthesis of an Xaa-Thr($\psi^{Me,Me}$pro) dipeptide and the two conformers accessible by rotation about the Xaa-Thr amide bond.](image)

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Results and Discussion

In order to investigate the cis–trans isomerization of the peptide bond in differently protected \(\Psi^{\text{Me,Me}}\)-pro-containing dipeptides, we prepared several derivatives 1–14 (Fig. 2) with either full protection (both \(N\)- and \(C\)-termini), \(N\)-terminus protection or uncapped \(N\)- and \(C\)-termini. We positioned Val, Phe, and Gly in the \(X\)aa position to examine the effect the \(X\)aa side chain had on the cis–trans ratio of the \(X\)aa-\(\Psi^{\text{Me,Me}}\)-pro amide bond. We included four \(\alpha\)-allo-threonine derivatives (5, 6, 9, and 12) because, while the influence of inverting the relative stereochemistry at the \(\alpha\)-position of the residue preceding a Thr(\(\Psi^{\text{Me,Me}}\)-pro) has been reported,[4] the effect of changing the relative stereochemistry between the \(\alpha\)- and \(\beta\)-positions on Thr(\(\Psi^{\text{Me,Me}}\)) formation and conformation has not been previously investigated.

Our standard conditions for the synthesis of \(X\)aa-Thr(\(\Psi^{\text{Me,Me}}\)-pro) derivatives involve treatment of the Thr-containing dipeptides with 2-methoxypropene at 0° C in the presence of an acid catalyst[13,14] Compounds 1–4 were readily synthetized from the corresponding fully protected dipeptides according to this general method. In the case of the \(\alpha\)-allo-Thr derivatives 5 and 6, this method gave only low yields of the corresponding pseudoprolines, but these were substantially improved using more forcing conditions (2,2-dimethoxypropene, pyridinium \(p\)-toluenesulfonate (PPTS), toluene, 80° C). Hydrolysis of the methyl esters of 1, 2, and 5, and hydrogenolysis of the benzyl ester of 6 were performed under standard conditions[13,14] to yield the carboxylic acids 7–9 and 12, respectively. Hydrogenolysis of the benzoxycarbonyl (Cbz)-groups of 7 and 8 was performed under standard conditions[13,12] to give 13 and 14, respectively. Compounds 10 and 11 were prepared according to the method of Mutter et al.[13]

We determined the cis/trans ratios of the amide bonds in dipeptides 1–14 using NMR spectroscopic techniques. In the fully protected dipeptides 1–4, a major and minor set of resonances were clearly observed in both the \(^1\)H and \(^{13}\)C NMR spectra, indicating the presence of two conformers in slow exchange (Fig. 3). In all cases, the major conformer was determined to be that with a cis-amide bond by the presence of typical nuclear Overhauser effect (NOE) cross peaks observed by 2D NMR rotating frame Overhauser effect spectroscopy (ROESY) and nuclear Overhauser effect correlation spectroscopy (NOESY) experiments (i.e. \(\alpha\)H\(_1\)–\(\alpha\)H\(_3\) and \(\alpha\)H\(_2\)–\(\beta\)H\(_1\) crosspeaks) that reflect the spatial proximity of the \(\alpha\)H\(_1\) and \(\alpha\)H\(_3\) protons in the cis-form.[16] The cis/trans ratios are given in Table 1. NMR spectra of a representative dipeptide 1 were obtained in a variety of

### Table 1. Ratios of cis/trans conformers as determined by \(^1\)H NMR

<table>
<thead>
<tr>
<th>Dipeptide</th>
<th>Compound no.</th>
<th>Solvent</th>
<th>cis/trans ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>CbzNH-Val-(\Psi^{\text{Me,Me}})Thr-OMe</td>
<td>1</td>
<td>CD(_3)CN</td>
<td>85:15</td>
</tr>
<tr>
<td>CbzNH-Val-(\Psi^{\text{Me,Me}})Thr-OMe</td>
<td>1</td>
<td>[D(_6)]DMSO</td>
<td>85:15</td>
</tr>
<tr>
<td>CbzNH-Val-(\Psi^{\text{Me,Me}})Thr-OMe</td>
<td>1</td>
<td>CDCl(_3)</td>
<td>85:15</td>
</tr>
<tr>
<td>CbzNH-Val-(\Psi^{\text{Me,Me}})Thr-OMe</td>
<td>1</td>
<td>CD(_2)OD</td>
<td>85:15</td>
</tr>
<tr>
<td>CbzNH-Phe-(\Psi^{\text{Me,Me}})Thr-OMe</td>
<td>2</td>
<td>CD(_3)CN</td>
<td>85:15</td>
</tr>
<tr>
<td>CbzNH-Gly-(\Psi^{\text{Me,Me}})Thr-OMe</td>
<td>3</td>
<td>CDCl(_3)</td>
<td>75:25</td>
</tr>
<tr>
<td>FmocNH-Phe-(\Psi^{\text{Me,Me}})Thr-OTce</td>
<td>4</td>
<td>CD(_3)CN</td>
<td>85:15</td>
</tr>
<tr>
<td>CbzNH-Val-(\alpha)-allo-(\Psi^{\text{Me,Me}})Thr-OMe</td>
<td>5</td>
<td>CDCl(_3)</td>
<td>95:5</td>
</tr>
<tr>
<td>FmocNH-Val-(\alpha)-allo-(\Psi^{\text{Me,Me}})Thr-OMe</td>
<td>6</td>
<td>CDCl(_3)</td>
<td>90:10</td>
</tr>
<tr>
<td>CbzNH-Val-(\Psi^{\text{Me,Me}})Thr-OMe</td>
<td>7</td>
<td>CD(_3)CN</td>
<td>80:20</td>
</tr>
<tr>
<td>CbzNH-Phe-(\Psi^{\text{Me,Me}})Thr-OMe</td>
<td>8</td>
<td>CD(_3)CN</td>
<td>80:20</td>
</tr>
<tr>
<td>CbzNH-Val-(\alpha)-allo-(\Psi^{\text{Me,Me}})Thr-OMe</td>
<td>9</td>
<td>CDCl(_3)</td>
<td>80:20</td>
</tr>
<tr>
<td>FmocNH-Val-(\Psi^{\text{Me,Me}})Thr-OMe</td>
<td>10</td>
<td>CD(_3)CN</td>
<td>&gt;95:&lt;5</td>
</tr>
<tr>
<td>FmocNH-Val-(\alpha)-allo-(\Psi^{\text{Me,Me}})Thr-OMe</td>
<td>11</td>
<td>CD(_3)CN</td>
<td>&gt;95:&lt;5</td>
</tr>
<tr>
<td>H(_2)N-Val-(\Psi^{\text{Me,Me}})Thr-OMe</td>
<td>12</td>
<td>CD(_3)OD</td>
<td>&gt;95:&lt;5</td>
</tr>
<tr>
<td>H(_2)N-Phe-(\Psi^{\text{Me,Me}})Thr-OMe</td>
<td>13</td>
<td>CD(_3)CN</td>
<td>65:35</td>
</tr>
<tr>
<td>H(_2)N-Phe-(\Psi^{\text{Me,Me}})Thr-OMe</td>
<td>14</td>
<td>CD(_3)CN</td>
<td>90:10</td>
</tr>
</tbody>
</table>
solvents (CD$_2$CN, CDCl$_3$, [D$_6$]toluene, [D$_8$]DMSO, CD$_3$OD) and at varying temperatures (300–370 K in [D$_8$]toluene) but the ratio of cis:trans conformers did not change significantly under these conditions. Substitution of the N-terminal Val with Phe (2) did not affect the ratio of cis:trans, but a slightly lower proportion of cis-amide conformer (75:25) was observed for the Gly-containing dipeptide 3. This is consistent with the proposal that steric interactions between the methyl groups of the ψMe,MePro and the side chain (or peptide backbone in the case of Gly) of the preceding amino acid are predominantly responsible for the favoured cis-conformation of these peptides.[3] Dipeptide 4, with alternative N- and C-terminal protecting groups (9-fluorenlymethoxy carbonyl (Fmoc)- and trichloroethyl ester, respectively) had an identical cis:trans ratio to the Cbz-, methyl ester protected analogue 2.

For the ψ-allo-Thr derivatives 5 and 6, a major set of resonances was observed in the $^1$H and $^1$C NMR spectra, with a second set of resonances from a second conformer present but difficult to distinguish owing to their low intensity and signal overlap. The observed major conformers were identified as those having a cis-amide bond by the presence of αH$_1$–αH$_2$. NOE crosspeaks observed by 2D NMR ROESY and NOESY experiments (Fig. 4). Despite the differences in stereochemistry with the previously discussed systems, an analysis of CPK models indicates that in 5 and 6, the αH$_1$ and αH$_2$ protons in the cis-conformer are in close proximity, whereas those in the trans-conformer are much further away from each other, so an NOE interaction is likely to be observed only for the conformer with a cis-amide bond. This proximity in the cis-conformer is evident in the X-ray structure of the carboxylic acid derivative 12 (see below).

Removal of the C-terminal protecting groups resulted in some changes in the cis:trans ratios. For the Cbz-protected dipeptides 7–9, a slightly lower amount of the cis-conformer was observed than for the analogous methyl esters. More notably, for the dipeptides bearing Fmoc protecting groups, ester hydrolysis resulted in significant increases in the amount of cis-conformer present, with only a single (cis) conformer observed by $^1$H and $^1$C NMR for 10, 11 and the ψ-allo-Thr derivative 12.

In two cases, the effect of deprotection of the N-terminus on dipeptide conformation was also investigated. Fully deprotected dipeptides 13 and 14 had significantly different ratios of cis:trans conformers in comparison with the fully protected analogues 1 and 2. In contrast to the results observed for the fully protected analogues and N-protected peptides where the side chain of the Xaa amino acid had little effect on the ratio of amide bond conformers, for the fully deprotected dipeptides, the cis:trans ratio depends significantly on the side chain of the N-terminal amino acid, with the Val derivative 13 having a significantly lower proportion of the cis-conformer than the Phe derivative 14. This difference cannot easily be explained by steric interactions, although the increased proportion of cis-conformer in 14, which has an aromatic side chain, reflects a similar propensity for Xaa-Pro dipeptides that has been attributed to stabilization of the cis-conformer as a result of CH–π interactions between the aromatic side chain and the protons at the α-position of the proline ring.[17] A similar interaction between the protons of the Thr(ψMe,MePro) methyl groups and the aromatic side chain of the Phe residue would provide a similar stabilization and may explain the observed differences between 13 and 14, although we did not observe any evidence for such an interaction.

In all 14 Xaa-Thr(ψMe,MePro) dipeptides that we examined by NMR spectroscopy, the cis-conformer was predominant, with cis:trans ratios varying from 65:35 to >95:<5. For dipeptides bearing N-protecting groups, this ratio did not significantly depend on the side chain of the Xaa residue or the stereochemistry of the Thr residue. However, it was affected by the presence of protecting groups at the N- and C-termini, suggesting that studies of longer peptides are required to provide a better understanding of the influence of the side chain on the conformation of Thr(ψMe,MePro) peptides.

### Solid-State Studies

Although several solution-phase studies of (ψ$^{R,R}$-pro)-containing peptides have been performed, little is known about the conformations of these peptides in the solid state. To the best of our knowledge, only one X-ray structure of a (ψ$^{Me,Me}$-pro)-containing dipeptide has been reported previously, indicating that Fmoc-Ala-Cyst(ψ$^{Me,Me}$-pro)-OH retains the cis-amide conformation in the solid state, although the amide bond is slightly twisted ($\omega_1 = -9.7^\circ$).[18] To obtain further information about the conformations of our Xaa-Thr(ψ$^{Me,Me}$-pro) dipeptides, we investigated the solid-state structures of three compounds.

Colourless prismatic crystals of 1 suitable for X-ray crystallography were grown by slow diffusion of diethyl ether into a methanolic solution (see Accessory Publication for ORTEP plot). The X-ray analysis indicates that in the solid state, the amide bond preceding the Thr(ψ$^{Me,Me}$-pro) adopts a trans-conformation (Fig. 5). This is in contrast to the NMR experiments, which indicate that in the solution-phase, the cis-conformer predominates (cis:trans 85:15) independently of solvent or temperature. In the solid state, the amide bond (N(1)–C(9)) is 1.3517(13) Å in length, which is similar to the length of the bond between the Val nitrogen atom and the Cbz protecting group (N(2)–C(14) = 1.3474(14) Å). The amide bond is slightly twisted ($\omega_3 = 171.36(11)^\circ$), indicating the steric hindrance that

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**Fig. 4.** 400 MHz nuclear Overhauser effect (NOE) correlation spectrum of 6 indicating the NOE interaction between the Val α-H (3.83 ppm) and the ψ-allo-Thr(ψ$^{Me,Me}$-pro) α-H (4.95 ppm). Small baseline peaks are attributable to the trans-conformer.
would result between the Thr(ψMe,MePro) methyl substituents and the Val side chain if it were planar.

An analysis of the crystal packing shows a strong carbamate (N(2)) to Thr(ψMe,MePro) oxygen (O(1)) hydrogen bond (see Table 2) as the most significant motif present within the lattice. This interaction results in the formation of infinite one-dimensional polymeric chains of molecules that propagate down the crystallographic b-axis. A section of one of these chains is shown in Fig. 5. Given the energy difference between the cis- and trans-conformers of ψMe,MePro dipeptides is \( \sim 62-75 \text{ kJ mol}^{-1} \),\(^{[10]} \) the stabilization introduced in the solid state by this hydrogen-bonding interaction may explain the differences observed between the solution-phase and solid-state conformations of this dipeptide.

Colourless plate-like crystals of 7, the free-acid analogue of 1, were isolated by slow diffusion of diethyl ether into a methanol solution and subjected to a crystallographic study (see Accessory Publication for ORTEP plot). The X-ray analysis revealed that 7 adopts a conformation \( (\omega) = 174.27(17)^{\circ} \) in the solid state that is almost identical to that of 1 (Fig. 6), again in contrast to the solution-phase conformation of this molecule. In fact, both 1 and 7 crystallize in the same chiral space group (orthorhombic \( P2_12_12_1 \)) with similar unit cells. In both structures, the a and b axis lengths are comparable, while the c axis lengths differ by only 2.1 Å. Given their chemical similarity, their structural similarity is not surprising, but suggests the possibility of use of species such as these in crystal engineering studies. Such a possibility is further demonstrated by the analysis of the crystal packing.

![Fig. 5. A schematic representation of the one-dimensional polymeric chain formed in 1. Dashed lines indicate hydrogen-bonding interactions.](image)

![Fig. 6. A schematic representation of the one-dimensional polymeric chain formed in 7 through carbamate-oxygen hydrogen bonds. Dashed lines indicate hydrogen-bonding interactions.](image)

<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>N(2)</td>
<td>H(2N)</td>
<td>0.850(14)</td>
<td>2.221(15)</td>
<td>3.0534(13)</td>
<td>166.1(13)</td>
</tr>
<tr>
<td>7</td>
<td>H(2O)</td>
<td>H(2O)</td>
<td>0.83(3)</td>
<td>1.87(3)</td>
<td>2.684(2)</td>
<td>164(3)</td>
</tr>
<tr>
<td></td>
<td>N(2)</td>
<td>H(2N)</td>
<td>0.82(3)</td>
<td>2.34(3)</td>
<td>3.14(3)</td>
<td>168(2)</td>
</tr>
<tr>
<td>12</td>
<td>N(2)</td>
<td>H(1N)</td>
<td>0.89(5)</td>
<td>1.88(5)</td>
<td>2.760(3)</td>
<td>171(4)</td>
</tr>
<tr>
<td></td>
<td>N(4)</td>
<td>H(2N)</td>
<td>0.81(4)</td>
<td>2.32(4)</td>
<td>3.066(3)</td>
<td>152(4)</td>
</tr>
</tbody>
</table>

Symmetry operators: \( A - x, y + 1/2, -z + 1/2; B - x + 1/2, -y, z + 1/2; C^T - y, -y, -z + 2/3 \).
Much like 1, molecules of 7 pack along the crystallographic b-axis to form a one-dimensional polymeric chain through carbamate (N(2)) to pseudoproline oxygen (O(1)) hydrogen-bonding interactions (see Table 2). A portion of this chain is shown in Fig. 6.

The presence of the free carboxylic acid group in 7 (compared with 1) adds an additional site for hydrogen bonding. Although this site does bind to the corresponding carboxylic site in adjacent molecules, unexpectedly, it does not bind in the classic carboxylic acid dimer and instead forms a polymeric chain (see Fig. 7), which propagates along the crystallographic a-axis at ~90° to the amide–oxygen hydrogen-bonding chain. Overall, these two sets of hydrogen-bonding interactions combine to form infinite two-dimensional sheet-like arrays that stack parallel to the crystallographic ab-plane.

The d-allo-Thr derivative 12 also crystallizes in the orthorhombic P2₁2₁2₁ space group. Colourless plate-like crystals were grown by the slow evaporation of a chloroform solution and there are two chloroform solvent molecules present in the lattice for each peptide molecule. In contrast to the previous two structures, in this case the amide bond has the expected cis geometry (ω₁ = −1.7(5)°). The structure is given in Fig. 8 and illustrates the short distance between the two α-protons (H(2) and H(9)) observed in the NOESY spectrum of this molecule.

Once again, the dominant crystal-packing effects are hydrogen-bonding interactions. However, in contrast to 1 and 7, these do not involve the Thr(ψMe,Me-pro) oxygen. Interestingly, again despite the presence of a carboxylic acid, the dimeric carboxyl acid motif is not present. Instead the carboxylic acid binds to the carbamate (N(2)) nitrogen and amide (O(4)) oxygen of the Val residue (Table 2), forming an infinite one-dimensional polymer (Fig. 9) along the a-axis. There is also a weak methyl–π interaction within the lattice. There are several non-classical hydrogen-bonding interactions present in the lattice, with the chloroform solvent molecules acting as H-bond donors and oxygen atoms as acceptors (Fig. 10). In particular, the presence of an H-bond between chloroform and the Thr(ψMe,Me-pro) oxygen (O(3)) is notable, as this prevents the formation of the carbamate to Thr(ψMe,Me-pro) oxygen H-bonds that are the predominant crystal packing interactions in 1 and 7. This may explain the conformational differences observed for 12 (cis-amide) in comparison with 1 and 7 (trans-amide) in the solid state. It is notable that no solvent is present in the crystal lattices of 1 and 7, despite their crystallization from methanol, a solvent that is more likely to form H-bonds than chloroform.

Conclusions

In solution, the predominant conformer for Xaa-Thr(ψMe,Me-pro) dipeptides is that in which the amide bond between the Xaa and Thr(ψMe,Me-pro) residues adopts a cis geometry. For dipeptides bearing both N- and C-terminal protecting groups, the ratio of cis:trans conformers does not depend strongly on solvent, temperature or the size of the Xaa side chain. It is notable that the relative stereochemistry of the Thr(ψMe,Me-pro) residue does not have a significant impact on the cis:trans amide bond ratio, with d- allo-Thr-containing peptides having similar (or enhanced) conformational preferences for the cis-isomer to the 1-Thr derivatives in solution. It has previously been shown that the relative stereochemistry between the Xaa and ψMe,Me-pro α-carbons does not have a significant influence on the amide bond conformation.[4] Minor differences in the cis:trans ratios are observed for N-protected dipeptides with a free carboxylic acid, depending on whether the N-protecting group is Cbz or Fmoc, with Fmoc-protected dipeptides having a higher proportion (>95%) of cis-conformer. However, for dipeptides with free N- and C-termini, the cis:trans ratio is strongly influenced by the Xaa side chain, with 14, which has an aromatic side.

Fig. 7. A schematic representation of the one-dimensional polymeric chain formed via carboxylic acid–carboxylic acid hydrogen bonds in 7. Dashed lines indicate hydrogen-bonding interactions.
Fig. 8. An ORTEP representation of 12 shown with 50% probability ellipsoids. Chloroform solvent molecules omitted for clarity.

Fig. 9. A schematic representation of the one-dimensional polymeric chain formed in 12. Dashed lines indicate hydrogen-bonding interactions. Val side chains and Thr(ψMe,Me-pro) methyl groups have been removed for clarity.

Fig. 10. A schematic representation of 12 illustrating the H-bonding interactions observed with CHCl₃ solvent molecules.

systems to avoid any influence of the protecting groups on conformation.

In the solid state, 1 and 7 adopt a trans-amide bond conformation, although in both cases the amide bond is slightly twisted. This directly contrasts with their solution structures and can be attributed to crystal-packing effects, with X-ray structures of both 1 and 7 showing a predominant carbamate (N) to Thr(ψMe,Me-pro) oxygen hydrogen-bonding interaction in the crystal lattice. The stabilization provided in the solid state by this hydrogen-bonding interaction and other crystal lattice effects is clearly greater than the 62–75 kJ mol⁻¹ stabilization of the cis-conformer relative to the trans-conformer in solution.¹⁰

In contrast, 12 adopts a cis-amide bond in both solution and the solid state. In this case, the co-crystallized chloroform solvent molecules act as hydrogen bond donors to the ψ-allo-Thr(ψMe,Me-pro) oxygen hydrogen-bond acceptor, preventing the formation of the stabilizing carbamate–pseudoproline oxygen hydrogen bonds observed for 1 and 7. An investigation
of the solution and solid-state structures of longer ψMeMe-pro-containing peptides and the relationship between peptide conformation and head-to-tail cyclization yields is ongoing in our laboratories.

**Experimental**

**Synthesis**

Preparative column chromatography was carried out using a Waters flash chromatography equipment under previously reported conditions [13] and used without protection from purification for 1H NMR studies.

Dimethoxypropane (1.00 mL, 8.14 mmol) and pyridinium-dimethoxypropane (472 μL, 3.85 mmol) and pyridinium-p-toluenesulfonate (60.0 mg, 0.231 mmol) were added to a solution of Fmoc-Val-α-Val-OMe (200 mg, 0.350 mmol) was dissolved in 10 mL of dry THF and 0.3% Pd/C catalyst was added. The reaction flask was purged with H2 and evacuated three times and the solution was left stirring under an atmosphere of H2 for 48 h. The solution was then filtered through a pad of Celite and the solvent removed under reduced pressure.

The crude product was purified by flash chromatography (2:1 v/v hexane/EtOAc) to give a yellow oil (329 mg, 75%). [α]D +29° (c 0.92 in CHCl3). δH (400 MHz, CDCl3) 7.76 (2H, d, J 7.6, ArH), 7.57 (2H, d, J 7.6, ArH), 7.42–7.28 (9H, m, ArH), 5.25 (1H, d, J 9.1, Val(NH)), 5.26 (1H, d, J 12.3, Cbz(CH2)), 5.16 (1H, d, J 12.3, Cbz(CH2)), 4.95 (1H, d, J 6.0, ThrΨ(α-CH)), 4.37 (3H, m), 4.22 (1H, t, J 6.8, Fmoc(Ch)), 3.83 (m, 1H, Val(α-CH)), 1.86 (1H, m, Val(β-CH)), 1.74 (3H, s, Me), 1.59 (3H, s, Me), 1.22 (2H, d, J 6.7, ThrΨ(γ-CH3)), 0.83 (3H, d, J 6.7, Val(γ-CH3)), 0.71 (3H, J 6.7, Val(γ-CH3)), 0.57 (1H, d, J 6.7, Val(γ-CH3)), 0.57 (m, 1H, Val(γ-CH3)), 0.57 (1H, d, J 6.7, Val(γ-CH3)), 0.57 (1H, d, J 6.7, Val(γ-CH3)), 0.57 (1H, d, J 6.7, Val(γ-CH3)), 0.57 (1H, d, J 6.7, Val(γ-CH3)), 0.57 (1H, d, J 6.7, Val(γ-CH3)).

**Fcoc-Val-α-Val-Thr(ψMeMe-pro)-OMe 8**

Dimethoxypropane (472 μL, 3.85 mmol) and pyridinium-p-toluenesulfonate (60.0 mg, 0.231 mmol) were added to a solution of Fmoc-Val-α-Val-OMe (408 mg, 0.769 mmol) dissolved in toluene (10 mL) and the mixture was heated at reflux for 16 h. The solution was cooled to ambient temperature before diluting with EtOAc (100 mL). The solution was then washed with saturated aqueous NaHCO3 (100 mL), then the aqueous layer was re-extracted with EtOAc (2 × 50 mL). The combined organic layers were then washed with brine (2 × 100 mL), dried (Na2SO4), and the solvent removed under reduced pressure. The crude product was purified by flash chromatography (2:1 v/v hexane/EtOAc) to give the title compound 6 as a yellow oil (329 mg, 75%). [α]D +29° (c 0.92 in CHCl3). δH (400 MHz, CDCl3) 7.76 (2H, d, J 7.6, ArH), 7.57 (2H, d, J 7.6, ArH), 7.42–7.28 (9H, m, ArH), 5.25 (1H, d, J 9.1, Val(NH)), 5.26 (1H, d, J 12.3, Cbz(CH2)), 5.16 (1H, d, J 12.3, Cbz(CH2)), 4.95 (1H, d, J 6.0, ThrΨ(α-CH)), 4.37 (3H, m), 4.22 (1H, t, J 6.8, Fmoc(Ch)), 3.83 (m, 1H, Val(α-CH)), 1.86 (1H, m, Val(β-CH)), 1.74 (3H, s, Me), 1.59 (3H, s, Me), 1.22 (2H, d, J 6.7, ThrΨ(γ-CH3)), 0.83 (3H, d, J 6.7, Val(γ-CH3)), 0.71 (3H, J 6.7, Val(γ-CH3)), 0.57 (1H, d, J 6.7, Val(γ-CH3)), 0.57 (1H, d, J 6.7, Val(γ-CH3)).
5.78 (2H, d, J 7.9, ArH), 7.4–7.3 (4H, m, ArH), 5.05 (1H, d, J 6.1, Thr(α-CH)), 4.45 (1H, m, Thr(β-CH)), 4.40 (2H, d, J 6.8, Fmoc(CH2)), 4.24 (1H, t, J 6.8, Fmoc(CH)), 3.80 (1H, m, Val(α-CH)), 1.87 (1H, m, Val(β-CH)), 1.77 (3H, s, Me), 1.61 (3H, s, Me), 1.38 (3H, d, J 6.0, Thr(γ-CH)), 0.94 (9H, d, 6.6, Val(γ-CH)), 0.88 (3H, d, J 6.6, Val(γ-CH)). 100 MHz, CDCl3) 173.3, 170.0, 156.6, 143.6, 141.3, 127.7, 127.1, 125.1, 120.0, 96.5, 72.4, 67.2, 63.4, 59.4, 47.0, 31.6, 24.8, 23.5, 19.4, 18.1, 15.2. m/z (EI, MNa+) Calc. for C29H34Cl6N2O6, Na 503.2158; found 503.2161.

Crystallography

Data were collected with μ scans to ~56° 2θ using a Bruker SMART 1000 diffractometer employing graphite-monochromated MoKα radiation generated from a sealed tube (0.71073 Å) at 150(2) K. Data integration and reduction were undertaken with SAINT and XPREP. Subsequent computations were carried out using the reXsan, WinGX-32, and XTAI graphical user interfaces. Structures were solved by direct methods using SIR97. Multiscan empirical absorption corrections were applied to the dataset using the program SADABS. Gaussian adsorption corrections were applied with XPREP. Data were refined and extended with SHELX-97. In general, non-hydrogen atoms with occupancies greater than 0.5 were refined anisotropically. Carbon-bound hydrogen atoms were included in idealized positions and refined using a riding model. Oxygen- and nitrogen-bound hydrogen atoms were first located in the difference Fourier map before refinement. Where these hydrogen atoms could not be located, they were not modelled.

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References

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